Standard Operating Procedure for the Analysis of (Tri-n-butyl)-n-tetradecylphosphonium chloride (TTPC) in Water by Multiple Reaction Monitoring Liquid Chromatography/Mass Spectrometry (LC/MS/MS)

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1. Scope and Application

1.1. This procedure covers the analysis of (Tri-n-butyl)-n-tetradecylphosphonium chloride (TTPC) in a water matrix by dilution with acetone, filtration and followed by liquid chromatography/tandem mass spectrometry analysis. TTPC is a biocide. The samples and standards are prepared in 75% acetone/ 25% water because TTPC has an affinity for surfaces and particles which is more pronounced at lower concentrations. The reporting range for this method is 100-4,000 ng/L.

1.2. This is the Chicago Regional Laboratory (CRL) method for the analysis of TTPC in water samples by LC/MS/MS. A standard multi-lab validated reference method does not exist.

1.3. The method detection limit (MDL) and reporting range for TTPC are listed in Table 1 (Appendix). This standard operating procedure (SOP) has been tested on ASTM Type I water and Chicago River water. The precision and accuracy (P&A) quality control acceptance criteria are listed in Table 2 (Appendix). Table 9 in the Appendix display the recoveries in ASTM Type 1 water and river water. MDL and P&A samples were analyzed in July 2014 (CRL work order 1408005). All site sample results are not reported below the reporting limit using this method. Reporting Limit Check Samples (RLCS) results may be reported below the reporting limit because they are spiked at or near the reporting limit.

1.4. P&A values will be updated regularly as more data is collected. The RL for a specific water sample may differ from that listed depending on the nature of the interferences in the sample matrix. Variability in historical LCS spike recovery may be used to estimate uncertainty, Table 2 (Appendix), in the measured concentrations of the target analyte generated with this SOP. Uncertainty estimates based on variability in LCS recovery are conservative, because some sources of variability are not included, such as subsample variability and matrix analyte recovery.

2. Summary of Method

2.1. A sample (2.5 mL) is transferred to a VOA vial, spiked with TTPC surrogate (not available at this time, a surrogate is being prepared and will be incorporated into this method) and target TTPC compound (only for laboratory control and matrix spike samples), add 7.5 mL of acetone and hand shaken for 1 minute. The samples are then

filtered through a Whatman Puradisc 25 NYL Disposable Filter unit (Diameter 25 mm, 0.2 µm Nylon membrane syringe driven filter unit) and then analyzed by LC/MS/MS.

2.1.1. The target compound is identified by comparing the single reaction monitoring (SRM) transition and its confirmatory SRM transitions if correlated to the known standard SRM transition (Appendix Table 7). The retention time (RT) for the analyte of interest must also agree with the RT of the mid-level standard by ±5%. The target compound is quantitated using the SRM transition of the target compound utilizing external calibration. As an additional quality control measure, isotopically labeled TTPC surrogate will be used when it becomes available. Recoveries will be monitored; the percent recovery of each should fall within the control limits of the method. The final report issued for each sample lists the concentration of TTPC, if detected, or non-detect at the RL if not detected, in ng/L.

3. Abbreviations and Definitions

3.1. ABBREVIATIONS

ADOC	Analyst Demonstration of Capability
CAS	Chemical Abstract Service
CCC	Continuing Calibration Check
CD	Compact Disc
CRL	Chicago Regional Laboratory
EPA	U.S. Environmental Protection Agency
IC	Initial Calibration
IDOC	Initial Demonstration of Capability
LC	Liquid Chromatography
LCS	Laboratory Control Sample
LCSD	Laboratory Control Sample Duplicate
LIMS	Relational Laboratory Information Management System
MDL	Method Detection Limit
MI	Matrix Interference
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
MS/MSD	Matrix Spike/Matrix Spike Duplicate
MSP	Method Specific Parameter
NA	Not available
NIST	National Institute of Standards and Technology
NPDES	National Pollution Discharge Elimination System
PPT	Parts per Trillion

PPE	Personal Protective Equipment
PPM	Parts per Million
P&A	Precision and Accuracy
QA	Quality Assurance
QC	Quality Control
QMP	Quality Management Plan
REC	Percent Recovery
RL	Reporting Limit
RLCS	Reporting Limit Check Sample
RSD	Relative Standard Deviation
RT	Retention Time
RTS	Retention Time Shift
SOP	Standard Operating Procedure
SRM	Single Reaction Monitoring
SS	Surrogate Standard
TC	Target Compound
TCL	Target Compound List
TTPC	(Tri-n-butyl)-n-tetradecylphosphonium chloride
UPLC	Ultra Performance Liquid Chromatography
VOA	Volatile Organic Analysis

3.2. MDL- Method Detection Limit: The minimum concentrations of analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

3.3. RL- Reporting Limit: This is the lowest concentration reported by CRL except in the case of a special request.

3.4. Batch QC- All the quality control samples & standards included in an analytical procedure.

4. Health, Safety and Waste Handling

4.1. Users of this method should operate a formal safety program. Perform this procedure in accordance with the CRL Chemical Hygiene Plan located in CRL's (G) share drive.

4.2. **WARNINGS** - Health Hazards

The toxicity and carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound is treated as a health hazard.

From this viewpoint, exposure to these chemicals is reduced to the lowest possible level. Exposure to these chemicals should be reduced to the lowest possible level and the appropriate PPE should be utilized.

Review MSDS's for specific physical and health and hazards including appropriate PPE to be used. MSDS's may be accessed at: Not Responsive Not Responsive

4.3. Waste Handling and Pollution prevention

4.3.1. A green tag identifying the contents of the carboy is placed on the waste container. Attach the appropriate chemical waste label with the date of beginning collection before using the container.

4.3.2. Report all major spills according to the CRL Chemical Hygiene Plan.

4.3.3. All used vials shall be placed in the green tag labeled carboy for disposal.

4.3.4. Additional information on waste reduction is found in the CRL Chemical Hygiene plan. Analyses performed by this method will generate solid (Primarily the filter units after use) and liquid wastes that are potentially hazardous.

5. Cautions and Interferences

5.1. Interferences

5.1.1. Method interferences may be caused by contaminants in solvents, reagents, glassware, LC vials/caps, disposable pipettes and other apparatus that lead to discrete artifacts or elevated baseline in the selected ion current profiles.

5.1.2. Matrix interferences may be caused by contaminants from the sample, sampling devices or storage containers. The extent of matrix interferences will vary considerably from sample source to sample source, depending upon variations of the sample matrix.

5.2. Cautions

5.2.1. All reagents and solvents should be of pesticide residue purity or higher to minimize interference problems, preferably LC/MS Grade.

5.2.2. Contaminants have been found in not properly cleaned glassware and glass syringes. TTPC sticks to surfaces, if the glassware is not properly cleaned and rinsed with solvent, such as acetone, 2-propanol and acetonitrile, low ppt concentrations affecting the analysis may be found. All of these materials are routinely demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as the samples. If found, measures should be taken to remove the contamination or data should be qualified.

5.2.3. Automatic Pipettes with polypropylene tips are used with this method. **The use of glass syringes for standards preparation, spiking, and calibrations generated erratic results and should be avoided.** A thoroughly cleaned 10 or 20 mL hypodermic glass syringe with a Nylon filter unit is used to filter samples and has been shown to perform well. If it can be proven that glass syringes can be used with consistent results they may be used.

5.2.4. The procedure described in the glassware cleaning section should be followed to make glassware free from interferences.

6. Equipment and Supplies

The procurement and management of CRL equipment and supplies must follow CRL SOP GEN026. The vendors' equipment and/or part numbers are listed for the supplies and reagents below. Any equivalent equipment or supplies from any vendor may be used, mention of brand names or part numbers is for informational purposes only, and no endorsement is implied.

6.1. Equipment

6.1.1. Liquid Chromatograph (LC) System - A Waters Acquity H-Class UPLC® with flow through needle design.

6.1.2. Analytical column - An analytical column (Waters Acquity UPLC® BEH C18, 2.1×100 mm and 1.7 µm particle size, Waters part number 186002352) or an analytical column that will achieve adequate results that meet or exceed this method.

6.1.3. Mass Spectrometer (MS) System – A Waters Xevo TQ-S triple quadrupole mass spectrometer is used. A mass spectrometer capable of MRM analysis with fast enough cycle time to obtain at least 10 scans over a peak is needed with adequate sensitivity.

6.1.4. Data Backup Device - A data archival unit to archive data. All the lab generated data are stored on the primary server. In addition, the laboratory has capabilities to store and retrieve data using other devices such as the networked secure server, external drives and CD or DVD writers.

6.1.5. Data System - MassLynx™ must be interfaced to the LC/MS/MS that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program. QuanLynx™ or TargetLynx™ is used for all data quantitation.

6.2. Calibrated support equipment (see CRL SOP GEN026 for recertification frequency)

6.2.1. Adjustable volume pipettes (Certifiedsemi-annually) – 10, 20, 100, 200 and 1000 µL, and 5 and 10 mL.

6.2.2. Analytical balance (Certified annually) accurate to ±0.1% of sample mass with Class 3 or 4 weights to verify calibration.

6.3. Glassware and Miscellaneous Supplies

6.3.1. Glassware and supplies

6.3.1.1. Vials - 2-mL autosampler vials with pre-slit PTFE/silicone septa or equivalent (Source –Waters, Part Number 186000847C)
6.3.1.2. Gases - Ultra pure Argon and Nitrogen
6.3.1.3. Class A volumetric glassware
6.3.1.4. VOA vials-20, 40 or 60 mL (Source: Thermo Scientific, Catalog # SB36-0040 for 40 mL)
6.3.1.5. Pipette tips – Polypropylene pipette tips free of release agents or low retention coating of various sizes (Source - Eppendorf, Catalog # 022491997, 022492080, 022491954, 022491946 and 022491512)
6.3.1.6. Whatman Filter Unit- Puradisc 25 NYL, Catalog # 6750-2502.

6.3.1.7. Glass Hypodermic Syringe with Lock Tip that will fit with the filter units. (Perfectum Catalog # 5137 is for the 20 mL syringe, other volumes may be used.)

CAUTION: These syringes must be cleaned with copious amounts of warm tap water, distilled water and thoroughly rinsed with acetone, acetonitrile and 2 propanol between uses, especially when used for highly contaminated samples. Sonication in these solvents should also be implemented. After cleaning a batch of syringes (approximately 10), a 10-20 mL amount of 75% acetone/25% water should be rinsed through the syringes, collected and analyzed to check for TTPC contamination. If found above half the reporting limit repeat the process of cleaning the syringes and check for contamination again.

6.3.2. Glassware Cleaning Instructions

All glassware is cleaned according to CRL SOP GEN008. All glassware is washed in hot water with detergent such as powdered Alconox, Deto-Jet, Luminox or Citrojet, rinsed in hot water and rinsed with distilled water. The glassware is then dried in an oven at 300ºC for up to 1 hr (except volumetric glassware, which is air dried). All glassware is subsequently cleaned with an organic solvent such as acetone, methanol and acetonitrile.

7. Reagents and Standards

Items shown are for information purpose only, equivalent reagents and standards may be used. All reagents and solvents should be of pesticide residue purity or higher to minimize interference problems, preferably LC/MS Grade or equivalent. GEN026 should be followed for reagents & standards ordering instructions.

7.1. Solvents and reagents

7.1.1. Acetonitrile (CAS # 75-05-8, Source: Fisher Scientific, Catalog # A955-4).
7.1.2. Water, HPLC mass spectrometry pesticide quality, In-house ASTM Type I water of specification D1193)
7.1.3. Methanol (CAS# 67-56-1, Source: Fisher Scientific, Catalog # A456-4).
7.1.4. Isopropyl alcohol (CAS # 67-63-0, Source: OmniSolv, Catalog # PX1834-1)
7.1.5. Ammonium Acetate (CAS# 631-61-8, Source: Sigma Aldrich, Catalog # 372331)
7.1.6. Acetone (CAS# 67-54-1, Source: Fisher Scientific, Catalog # A929-4)

7.1.7. (Tri-n-butyl)-n-tetradecylphosphonium Chloride (CAS# 81741-28-8, Source: Alfa Aesar, Catalog # B24775.

7.1.8. Sodium Bicarbonate (CAS# 144-55-8, Source: Sigma-Aldrich, Catalog # S6014).

7.2. Reagents and standard preparation

All calibration standard preparations should be noted in the logbook and LIMS. All standard stock vials include the LIMS ID, name, concentration, date made, and initials of creator and are referenced in the logbook. All LC or small vials where space is an issue shall include a LIMS number which can be referenced back to the logbook. All concentrated stock solutions are prepared with acetone:water (75:25) unless otherwise stated.

Expiration time of a prepared standard is one year from the time prepared. The standards can be used for more than one year if they fall within +/- twenty percent from the calibration standard that is less than 1 year old. Presently there is no holding time study for this standard and the 1 year time period is an estimate with no laboratory data supporting this time period.

Label all standards with LIMS ID; and verify the correct grade of solvents in comment field. Traceability of standards is established using the manufacturer's specifications provided at time of purchase.

The specific instructions for the preparation of stock standards, spiking solutions and QC batch are listed below. The calibration levels (prepared from the high level calibration stock), method blank, laboratory control samples, matrix spike samples and duplicates are made for each batch of samples. They are usually prepared and analyzed immediately. CCC check standard is also prepared for each batch of samples.

7.2.1. TTPC Surrogate Spiking Solution

A surrogate spiking solution is not available at this time.

7.2.2. MS/MSD and LCS/LCSD Spiking Solution

Each 2.5 mL MS/MSD or LCS/LCSD sample is spiked with target TTPC (listed in section 7) to achieve a concentration of 2000 ng/L in the 2.5 mL of sample. For example, 50 µL of a target spike solution at 100 µg/L prepared in 75% acetone/25% water is added to the 2.5 mL quality control samples.

7.2.3. Reporting Limit Check Spiking Solution

The reporting limit check sample (RLCS) is prepared by spiking the RLCS with the target analyte near the reporting limit. For Example, a 25 µL volume of a reporting limit check spiking solution at 10 µg/L in 75% acetone/25% water (prepared by dilution from the 100 µg/L target spike solution) is added to the reporting limit check sample to prepare 100 ng/L of TTPC in a 2.5 mL sample.

7.2.4. Calibration Standards

Calibration stock standard solution A is prepared from the 100 µg/L target spike solution. 500 µL of the target spike solution is added to a 50 ml volumetric flask and made up to 50 ml volume with 75:25 acetone and water (Solution B). Stock standard solution A (Level 8 Appendix Table 4) containing 1000 ng/L of TTPC is diluted to prepare Levels 1 through 7 as shown in Table 3 and 4 (Appendix). All calibration standards should contain 75:25 acetone to water in 2 mL LC vials. The continuing calibration check (CCC) is a mid-level calibration standard, extra preparation is not required.

7.2.5. Target Spike Solution

The target spike solution is prepared by preparing a 100 µg/L solution in 75% acetone/25% water from a concentrated stock standard. The concentrated stock standard concentration can vary when preparing from neat material, usually between 50-100 mg/L TTPC in 75% acetone/25% water.

8. Sample Handling and Preservation

8.1. Sample Collection Criteria

Grab samples are collected in glass containers with Teflon™ lined caps. Field blanks are needed to follow conventional sampling practices. Surface binding may bias data. All samples are iced or refrigerated at <6°C from the time of collection until sample preparation. Conventional laboratory practices involving chain of

custody, field sampling, lab custody beginning with receipt and transfer custody, and sampling protocols are referenced from CRL SOP GEN013.

8.2. Sample Preservation and Storage

All samples are iced or refrigerated at < 6°C from the time of collection until sample preparation. At the laboratory, prepared and filtered samples are stored in the refrigerator at < 6°C at all times while not being analyzed. Holding times have not yet been established for this analyte in the various matrices. The prepared samples are normally analyzed the day of preparation or within 7 days if multiple dilutions and analysis are required. Samples are all labeled with a unique LIMS identifier which is tracked through the entire analysis process.

9. Sample Preparation and Analysis

9.1. Sample Preparation –Step I

Each batch of samples (20 or Less) shall contain at least a Method Blank, Laboratory Control Sample and Laboratory Control Sample Duplicate, Duplicate, Matrix Spike and Matrix Spike Duplicate (if enough sample is available) and a Reporting Limit Check Sample.

9.1.1. Method Blank and Reagent Blank

The method blank is prepared by measuring 2.5 mL of ASTM Type I water in a 40 mL VOA vial, and then taken through the sample preparation Step II in Section 9.2.

The reagent blank is a 75% acetone/25% water solution that is used to prepare the calibration levels and all dilutions.

9.1.2. Laboratory Control Sample/Laboratory Control Sample Duplicate

A 2.5 mL amount of ASTM Type I water is added to each of two 40 mL VOA vials. The samples are spiked with 50 µL of a 100 µg/L target spike solution and then taken through the sample preparation Step II in Section 9.2.

9.1.3. Reporting Limit Check

A 2.5 mL amount of ASTM Type I water is added to a 40 ml VOA vial. The sample is spiked with 25 µL of a 10 µg/L Reporting Limit Check solution and then taken through the sample preparation Step II in Section 9.2.

9.1.4. Sample and Sample Duplicate

A 2.5 mL aliquot of sample is added to each of two 40 ml VOA vial. The samples are taken through the sample preparation Step II in Section 9.2. **Note: If the samples are collected and acidified by error in the field, solid sodium bicarbonate may be added to neutralize to near neutral. This will adjust the volume negligibly compared to using a solution of sodium bicarbonate.**

9.1.5. Matrix Spike/Matrix Spike Sample Duplicate

A 2.5 mL aliquot of sample is added to two 40 ml VOA vials. The samples are spiked with 50 µL of a 100 µg/L TTPC target spike solution and then taken through the sample preparation Step II in Section 9.2.

9.2. Sample Preparation – Step II

9.2.1. To all samples, 7.5 ml of acetone is added to all samples and hand shaken/vortexed for ~ 1 minute.

9.2.2. All the samples are filtered through a Nylon filter unit using the cleaned glass syringe. **NOTE: It is important that this syringe is cleaned with water and organic solvents mentioned before use as described in Section 6.** The filter does not require rinsing prior to use. This chemical should not be found in facilities that manufacture filter units. An aliquot of that solution is transferred to a LC vial and a LC cap is applied. The final volume of the solution is 10 mL, for quantitation purposes.

9.2.3. The analyte concentration for all samples will be reported in ng/L

9.3. Analytical Procedure

9.3.1. Sample Analysis Procedure

Instrument conditions for LC/MS/MS are described in section 9.4.2. The target compound is identified by comparing the sample single reaction monitoring (SRM) transition to the known standard SRM transition. Two confirmatory transitions are available for TTPC (Appendix Tables 5 and 7). The retention time (RT) for the analyte of interest must also agree with the RT of the mid-level standard by ±5%. The target compound is quantitated using the SRM transition of the target compound utilizing external calibration. The final report issued for each sample lists total concentration of TTPC, if detected, or non-detect at the RL, if not detected, in ng/L.

If the absolute amount of a target compound in a sample exceeds the working calibration range the sample is diluted and re-analyzed. This should be done by diluting the sample with 75:25 acetone: water.

9.3.2. Qualitative and Quantitative Analysis

9.3.2.1 The quantitation of the target analyte is accomplished with QuanLynx™ or TargetLynx™ software. No internal standards are used. Refer to Table 5 for the MRM transitions and retention times. The MRM analysis provides confirmation by isolating the precursor ion, fragmenting it to the product ion, and also relating the transition to retention time data. The manual should be consulted in order to use the software correctly. The quantitation method is set as an external calibration using the peak areas in ppt units.

9.3.2.2 If there are two or more analyses for a particular fraction due to sample dilution, the analyst must determine which is best to report on the sample summary results sheet based on evaluation of all available data relevant to a given sample. The target compound is identified by comparing the sample primary SRM transition and its confirmatory SRM transitions if correlated to the known standard SRM transition. Confirmatory transitions are available (Appendix Tables 5 and 7). The primary/confirmatory SRM ion ratios shall meet the criteria set in the quantitation method by ± 30% of the average of the ratios calculated from the calibration levels on the day of analysis. This ratio will vary depending on the instrumental acquisition parameters. Due to sensitivity issues depending on the instrument used, some ion ratios may not meet the ± 30% ion ratio at the lower concentrations. If this is the case, the data should be qualified "J" and explained in the narrative. If a co-eluting peak is present that interferes with the primary transition a confirmatory transition may be used for quantitation, this change must be documented in

the case narrative. If a co-eluting peak interferes with a confirmatory transition this must be documented in the case narrative. These changes should be rare exceptions.

9.3.2.3 Regression fits should exclude the point of origin (X=0, Y= 0) and be weighted by 1/concentration in order to increase the accuracy of the curve at the lower concentrations. For linear regression to be used, the coefficient of determination, r^2, should be > 0.98 for each analyte, and for quadratic regression the r^2 should be >0.99. Upon inspection of the calibration curves, if one of the calibration standard injections, other than the high or low, skews the curve such that the r^2 is unacceptable, this point must be re-injected and replaced in the calibration curve or a new calibration curve must be made. If the low and/or high point is excluded, a six point curve is acceptable for quadratic fit and five point for a linear fit, but the calibration range and reporting limits must be modified to reflect this change.

9.3.2.4 The retention time window of the SRM transitions must be within 5% of the retention time of the analyte in a Level 4-6 calibration standard. If this is not true the calibration curve needs to be re-analyzed to see if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, the analyte is referred to as an unknown. If the retention time is drifting more than 5% this will be noticed in the end calibration check and shall be noted in the case narrative. There are 3 SRM transitions for TTPC in this method so a false positive is unlikely.

9.3.2.5 If there is no confirmatory transition, or it does not meet the ±30% acceptance criteria for the primary/confirmatory SRM area ratios (refer to Appendix Table 5), the analyte is listed as a non-detect or reported and explained in the narrative based upon the justifications in Section 9.3.2.2.

9.4. Instrument Calibration and Standardization Procedures

9.4.1. Calibration of Mass Spectrometer

The Waters Xevo TQ-S mass spectrometer is calibrated monthly or when mass shifts of more than 0.2 Dalton are noticed by the analyst. The calibration file is saved in the MassLynx™ file folder. The calibration solution normally used is

a mixture of phosphoric acid. Other calibration solutions can also be used per manufacturer's specifications. The detailed procedure for calibrating the mass spectrometer can be found in the MassLynx™ instruction manual located on the instrument computer.

9.4.2. Instrument Operating Conditions

Analytical conditions for liquid chromatography and mass spectrometer are described below.

9.4.2.1 Liquid chromatographic conditions:

9.4.2.1.1. Analytical Column: (Acquity UPLC® BEH C18, 2.1×100 mm, 1.7 µm particle size)

9.4.2.1.2. Injections of all standards and samples are normally made at a 50 µL volume. Other injection volume may be used to optimize conditions. Standards and samples must be in 75:25 acetone:water. In the case of extreme concentration differences amongst samples, it is wise to analyze a blank after a concentrated sample and before a dilute sample to minimize carryover of analytes from injection to injection. However, there should not be carry-over between samples; The H-Class UPLC® has a flow through LC needle design. The gradient conditions for liquid chromatography are shown in Table 6 (Appendix).

9.4.2.2 Mass spectrometer conditions:

Variable parameters for TTPC is shown in Table 7 (Appendix). The instrument is set in the Electrospray (+) positive source setting. The values for the following parameters are shown here for information only. The values on your instrument or a different vendors may be different. These conditions should be checked and optimized on the individual system.

Capillary Voltage: 1 kV
Cone: Variable depending on analyte
Extractor: 2 Volts
Source Temperature: 150 °C

Desolvation Gas Temperature: 500°C
Desolvation Gas Flow: 900 L/hr
Cone Gas Flow: 150 L/hr
Collision Gas Flow: 0.15 mL/min
Low Mass Resolution 1: 3
High Mass Resolution 1: 14
Ion Energy 1: 1
Entrance Energy: 1
Collision Energy: Variable depending on analyte
Exit Energy: 1
Low Mass Resolution 2: 2.8
High Mass resolution 2: 14
Ion Energy 2: 1
Gain: 1.0
Multiplier: 512.82
Inter-Scan Delay: 0.003 seconds

9.4.3. Calibration

9.4.3.1. Initial Calibration

The initial calibration contains an eight-point curve. Depending on instrument type, the sensitivity and calibration curve responses may vary. At a minimum, a five point linear or a six point quadratic calibration curve will be utilized for all analytes. A calibration curve and an instrument blank will be analyzed at the beginning of each run or daily to ensure instrument stability. A new curve will be generated daily. The calibration method is saved and used to quantify all samples. Acceptance limit for calibration curve is mentioned in sections 9.3.2.3 and 10.1.4.

9.4.3.2. Continuing Calibration Check (CCC) and/or End Calibration Check

Within 24 hours of the last injection of the previous initial calibration curve, a continuing calibration check standard at or near the midpoint of the calibration curve must be run. A QC acceptance criterion for CCC is included in section 10.2.6. A new calibration must be generated every 24 hours regardless of a passing CCC.

9.4.4. Auto Sampler Schedule/Analytical Sequence

Prepare a sequence that includes all QC samples and field samples. The first sample to be analyzed is a solvent reagent blank sample. The calibration standards levels are analyzed next. The next samples to be analyzed should be in the following recommended sequence: reagent blank, method blank, RLCS, LCS/LCSD, diluted samples, samples, duplicates, MS/MSD and CCC.

10. Quality Control

Refer to Region 5-Central Regional Laboratory SOP GEN028 and the CRL QMP for a detailed QA/QC protocol.

10.1. Minimum Requirement

10.1.1. An analysr must have an approved Analyst Demonstration of Capability (ADOC) prior to reporting data from a CRL method. The analyst who performs the Initial Demonstration of Capability (IDOC) will be considered to have met the ADOC requirement MDL with P&A studies must be performed before an analytical SOP may be used and repeated for any major SOP revisions. These studies evaluate whether the reporting limits and calibration standard concentrations are appropriate. MDL studies must be performed annually for each method if values are reported below the RL. A reporting limit check standard must be analyzed with each batch of 20 samples or less or 4 reporting limit check standards per year shall be analyzed.

10.1.2. Demonstration of Capability (DOC)

An initial demonstration of the laboratory capability to generate data of acceptable quality is made. A precision and accuracy (P&A) study must be performed whenever a major modification is made to this method. The average percent recovery (X), individual percent recovery and the standard deviation (σ) of the recoveries is calculated for each analyte. QC confidence limits at 99.7% (3σ) are established. Quality control acceptance criteria for P&A study is shown in Table 2 (Appendix). These limits are preliminary and based upon only six replicates, they will be updated as more data is generated.

For a precision and accuracy (P&A) study, 4 samples containing TTPC at 2000 ppt must be analyzed as replicates. These samples are then analyzed according to the method described in Sections 9.

10.1.3. Method Detection Limit Test (MDL)

The method detection limit test is performed in accordance with the definition and procedure outlined in 40 CFR Part 136, Appendix B. MDL data for this method is shown in Table 8 (Appendix). MDL studies will be repeated at least yearly if any data are reported between the MDL and RL.

10.1.4. Example Calculation of Sample Concentration Reported.

The concentration of sample is calculated using the equation 1.

Equation 1

$$\frac{V_f}{V_i}(C_u) = C_f$$

Where:

V_f = Final Volume

V_i = Initial Volume

C_u= Uncorrected Concentration

Cf = Final Concentration (Corrected for Dilution)

10.1.5. Calibration Range

The initial calibration contains an 8 point curve. Each batch contains a calibration curve. Depending on instrument type, the sensitivity and calibration curve responses may vary. At a minimum, a five point linear or a six point quadratic calibration curve will be utilized for all analytes. The coefficient of the determination (r^2) of the linear fit must be greater than or equal to 0.98. The coefficient of the determination (r^2) of the quadratic curve must be greater than or equal to 0.99. The calibration points used to generate curve should not deviate more than ±30 % from the curve.

10.2. QC Acceptance Limits and/or Criteria

All control limits are preliminary because this is a new SOP and collected data is insufficient to determine historical acceptance criteria over the course of a longer period. The control limits provided will be revised when more data is collected.

10.2.1. Matrix Spike

As part of the Region 5 CRL QC program, spike accuracy for each matrix is monitored and updated regularly. Records are maintained of spiked matrix analyses, the average percent recovery (X) and the standard deviation of the percent recovery is calculated. This procedure maintains a 99.7% confidence interval from X±3σ control limits for spike compounds. The acceptance criteria that should be used are shown in Table 2 (Appendix) until more matrix spike data is obtained.

Calculate the percent recovery of the spike (P) using Equation 2.

Equation 2

$$P = 100 \times \frac{SSR - SR}{SA}$$

Where:

SSR = MS/MSD Spiked Sample Result

SR = Unspiked Sample Result

SA = Spike Concentration

P =Percent Recovery

10.2.2. Duplicates

The Relative Percent Difference (Equation 3) for the duplicate sample should be <30% RPD. If greater the associated sample concentration is qualified estimated "J" and noted in the case narrative.

Equation 3

$$RPD = \frac{|[S] - [DS]|}{([S] + [DS]) / 2} \times 100$$

Where,

S= Concentration of the sample

DS = Concentration of the duplicate sample

10.2.3. Surrogates

Not available at this time.

10.2.4. Reagent Blank

A reagent blank is prepared with 75:25% acetone:water for every 20 samples to investigate for system/laboratory contamination. This solution is also used to make the calibration curve standards and to dilute samples if required. The concentration of target analyte in the blank shall be less than half the reporting limit.

10.2.5. Method Blank

A method blank for every 20 samples will be prepared in water and prepared as a sample to investigate for contamination during sample preparation and extraction. The concentration of target analytes in the blank shall be less than half the reporting limit or the data shall be qualified that there is a blank issue and the reporting limit shall be raised to at least 3 times above the blank contamination concentration.

10.2.6. MS/MSD

A matrix spike and matrix spike duplicate is extracted with each matrix at a frequency of at least one MS/MSD pair for every 20 samples to investigate for matrix interferences. If the laboratory has not received MS/MSD samples for site specific precision and accuracy (P&A) data, the site data quality will be evaluated solely on the Lab Control Sample criteria. Table 9 in the Appendix display recoveries in river water and ASTM Type 1 Water.

10.2.7. Continuing Calibration Check (CCC) and/or End Calibration Check

Analyze a mid-level continuing calibration standard at the end of each batch. All analytes should be within ±30% of their expected values. If not, a separately prepared CCC may be analyzed. If the second CCC fails the criteria, ±30% of the expected analyte value, the data may be reported qualified as estimated "J" or if sample and time is available be re-analyzed with a new calibration curve and end CCC check. If any data is reported with any failing QC it shall be explained in the case narrative and properly qualified. Calculate the percent Difference (%D) using Equation 4.

Percent Difference

The percent difference (%D) between the calculated and expected concentration in the continuing calibration verification standard is calculated using Equation 4.

Equation 4 :

$$\%D = \frac{\text{calculated concentration} - \text{expected concentration}}{\text{expected concentration}} \times 100$$

10.2.8. Reporting Limit Check Samples

Each batch or within the 24 hour analysis window a reporting limit check sample must be analyzed. The reporting limit check sample is processed like a Laboratory Control Sample just spiked at or near the reporting limit. This sample is to verify that if the analytes were present at the reporting limit they would be confidently identified. The recovery criteria for the reporting limit check sample is 86-113% based upon MDL criteria.

10.2.9. LCS/LCSD

As part of requirement of the Region 5 CRL QC program, spike accuracy for water is monitored with each batch. At least one LCS/LCSD pair for every

20 samples is extracted and analyzed. The percent recovery limits for the target compound is updated regularly based on historical recovery data and are based on a 99.7% confidence interval from X±3σ control limits. The acceptance criteria are shown in Table 2 (Appendix).

10.3. Immediate Corrective Actions

All control limits are preliminary because this is a new SOP and collected data is insufficient to determine acceptance criteria based upon historical data. The control limits will be revised when more data is collected. Exceedances of QC criteria need to be discussed in the case narrative.

10.3.1. Laboratory Control Samples

As part of the Region 5 CRL QC program, spike accuracy for ASTM Type I water is monitored with each batch. Records are maintained of spiked sample analyses, the average percent recovery and the standard deviation of the percent recovery is calculated. This procedure maintains a 99.7% confidence interval from X± 3σ control limits for spike compounds. Criteria for LCS/LCSD Samples are shown in Table 2 (Appendix): If one is high or low and one acceptable, no qualifier is added to the data. If one high and one low-"J" flag (estimated) is applied. If both high and the analyte is present "K" flag for high bias, if not present no qualifier is added. If both LCSs are low and the analyte is present apply an "L" flag (low bias), if not present a "J" flag. These criteria pertain to all samples.

10.3.2. Matrix Spike Samples

As part of the Region 5 CRL QC program, spike accuracy for each matrix sample is monitored with each batch. Records are maintained of spiked sample analyses and the average percent recovery and the standard deviation of the percent recovery is calculated. This procedure maintains a 99.7% confidence interval from X± 3σ control limits for spike compounds. Criteria for MS/MSD Samples are shown in Table 2 of the Appendix (LCS/LCSD Criteria). If one is high or low and one acceptable, no qualifier. If one high and one low-"J" flag. If both are high and the analyte present "K" flag for high bias; if not present no qualifier is added. If both low and analyte is present apply an "L" flag (low bias) and if analyte not present a "J" flag. If the MS/MSD are greater than 30% RPD,

the associated sample concentration is qualified estimated "J" and noted in the case narrative. These criteria pertain only to the native unspiked sample.

10.3.3. Method Blank and Reagent Blank Samples

The concentration of target analyte in the blank shall be less than half the reporting limit or the data shall be qualified (K, high bias) that there is possible interference from the blank. Or, the reporting limit for the associated sample(s) shall be raised to at least three times above the blank contamination concentration. Since a quadratic fit is often used, the concentrations below the reporting limit are not accurate. The response/area count of the blank should be less than half the response/area count in the associated sample(s).

10.3.4. Reporting Limit Check Samples

Each batch or within the 24 hour analysis window a reporting limit check sample must be analyzed. The reporting limit check sample is processed like a Laboratory Control Sample just spiked at or near the reporting limit. This sample is to check if the analytes were present at the reporting limit, they would be identified. The recovery criteria for the reporting limit check sample is 86-113% based upon MDL criteria. If the analytes are not present in the reporting limit check sample, the data for all non-detects is qualified "UJ" and "J" if detected up until a passing LCS concentration level. If the recovery is biased high, all non-detects in site samples are not qualified but if present are qualified "J" until a passing LCS concentration level. The QC failure is explained in the narrative accompanying the data.

10.3.5. Duplicate Samples

A duplicate sample is analyzed with every batch of 20 samples. The relative percent difference in the duplicate must be less than ±30%. If not the native sample is qualified estimated, "J".

10.3.6. Surrogates

10.3.6.1. No surrogates available at this time.

10.4. Corrective Actions

10.4.1. Corrective actions should follow the guidelines in the CRL QMP.

11. Data and Records Management

11.1. Calculations and Documentation

11.1.1. Computer programs used for analysis of data include MassLynx™ with QuanLynx™ or TargetLynx™ software. For additional information see section 11.2. Refer to Equation 1, Section 10.1.4, how final concentration is calculated.

11.1.2. Use of professional judgment in the data reduction and verification process must be documented. Manual integrations are documented according to the CRL QMP and CRL SOP GEN029. The default significant figures reported for this analysis is three.

11.1.3. All data packages are verified by a qualified analyst. The qualified analyst signs off on the checklist and the LIMS report to release the data.

11.1.4. All QA/QC data and final results are reported to the customer.

11.1.5. Electronic data storage reference: All data is archived according to CRL QMP.

11.2. Reporting Result Procedure

11.2.1. The concentration in the sample is calculated using the linear or quadratic calibration curve. The sample results are reported in nanograms per Liter (ng/L). These results are reported to the Reporting Limit without any corrections for recovery data. All QC data obtained are included in the CRL data packages.

11.2.2. Verify that all analytes have been properly identified and quantified in the chromatogram. Using software programs, manually integrate as necessary. The integration is recorded before and after in the MassLynx™ software clearly showing the before and after integration.

11.2.3. Review the report for calibration outliers and make area corrections for any peaks that are improperly integrated. If corrections have been made, update the calibration file and regenerate a calibration report. Alternatively, re-analyze "nonconforming" calibration level(s) and repeat the above procedures.

11.2.4. If the initial calibration data are acceptable, generate the calibration report. Samples may be analyzed during the 24-hour period after the last calibration standard injection, ending with injection at or near the midpoint of the calibration curve. After that 24-hour period is expired, a new calibration curve must be generated to continue analysis.

11.2.5. Archive all the raw data to the R5CRL server according to CRL QMP.

11.2.6. Generate quantitation reports for all samples following data system and CRL QMP. Generate the final data. If manual integration was used, the QuanLynx Audit Report can be consulted showing the details of how, when and why the peak was changed. The entire quantitation method is saved and archived.

11.2.7. Review the quantitation reports for all samples making sure all surrogate and target compounds have been properly quantitated. Check for integration errors.

11.2.8. Review the quantitation reports for all samples. Delete any false positive method specific parameters.

11.2.9. Create sample header and miscellaneous information files for all samples in the analytical sequence.

11.2.10. Generate a *.XML report using QuanLynx™ software.

i. Open QuanLynx document (*.qld).

ii. Go to *File* > *Export* > *XML…*

iii. Save file (*.xml extension).

11.2.11. Be sure the blank sample data have been properly reviewed.

11.2.12. Verify that all spike compounds were present on the MS/MSD and LCS/LCSD sample quantitation reports. Investigate any differences in spike concentrations.

11.2.13. Generate analysis data sheets for blanks and samples. Review the final results and upload the applicable data (sample results, surrogate spike compound recovery data and matrix precision and accuracy data) into CRL LIMS. Sufficient information should be present in the data package to indicate which QC samples are relevant for the field sample injections (copies of laboratory notebook pages, bench sheet(s), injection sequences, etc.).

11.2.14. On a daily basis, archive all the processed data files to their original directories in the R5CRL server.

11.3. Data Package Assembly

11.3.1. Create a calibration deliverables package with the following: copies of sequence files, initial and continuing calibration reports, and quantitation reports for calibration standards.

11.3.2. Create a QC deliverables package with applicable QC Forms.

11.3.3. Create a sample deliverables package with the following for each sample: sample data sheet, sample quantitation report. Make sure all method specific parameter peaks are correctly labeled on the chromatogram.

11.4. Data Package Quality Control

11.4.1. Analysts are primarily responsible for performing data evaluation and verification tasks. See the LC/MS data review, SOP GEN028, for validation and qualification criteria. For data qualification purposes, apply the QC criteria specified in this SOP.

11.4.2. All data packages must be approved and signed by the CRL Primary Analyst.

11.4.3. Documents pertaining to the original observations and data (including supportive data) generated by sample analysis shall be maintained in the final data package per LIMS work order. Pertinent print-out hard copies of

customer contact communication and/or non-conformance notifications are included in the relevant data package as supportive data.

11.5. Data Reduction and LIMS Data Entry and Reporting

11.5.1. All LIMS data entry is based on first creating a bench sheet describing the sample preparation. This bench sheet describes the samples prepared, and the quality control samples. The analyst must make certain that the preparation date in LIMS matches the actual preparation date. By convention, if the sample preparation proceeds overnight, the date started is used for the LIMS preparation date.

11.5.2. When the data are ready to be entered, the bench sheet is called up into the Data Entry/Review module. All analyses or selected analyses can be included. If only a few analyses are to be entered, data may be entered manually. Manual data entry has resulted in many errors, and should be avoided. Use DataTool or other automated data entry procedures when available.

11.5.3. When performing manual data entry, enter the results in the column **Result**. For each result, enter the date of analysis in the column **Analyzed**. This column has a calendar feature as do other date fields in LIMS. If dilutions were necessary for the analysis, enter the dilution factor in the column **Dilution**. The sample result should be the one measured and not corrected for the dilution factor. Verify that the correct initials are present in the Analyst field and the instrument field.

11.5.4. If all data are entered, click the **Save** button on the top row. After saving, proceed to the Review page by clicking **Query** on the second row. Verify that all conversions to reporting units and dilutions have been calculated correctly. Verify that reporting limits have been correctly applied. Flags may be added at this stage, following the guidance given in the Data Review SOPs. Before submitting the package for review, the data should be locked as soon as the data is merged and entered and the status updated to 'analyzed'.

11.5.5. Data Tool data entry: Once the batch is called up in Data Entry/Review, click **Export** to create an Excel file in the User Directory. Name this file in such a manner that it can be easily associated with that analysis.

11.5.6. A file created by the instrument generated by Quanlynx (Section 11.2.10), contains the instrument readings for the samples. If multiple measurements for a given sample are present, such as for dilutions, the .txt file can be loaded into a text editor, such as Word Pad, and the sample IDs for the sample readings that are not to be used can be altered so that Data Tool does not recognize them. There is no need to remove the analytical spikes, because the software recognizes them as such.

11.5.7. Once in Data Tool, click **Browse** for the Element Data Entry Table, and call up the .xls file created earlier. Click Browse for the Instrument Data File, and call up the file created earlier. If unneeded sample entries remain in the lower left-hand box, click **Clear**. Double-click on the desired epatemp.txt file and either Auto Select or highlight individual samples and click Include.

11.5.8. Once the samples and quality control samples are selected, click **Done** and it will return to the main **Data Tool** page.

11.5.9. Select the appropriate cross table on Main DataTool page:

(Example)

File
Select Cross Table…
G:\CRL Group - MS\data_upload\NP_OP_isomers
"DATATOOL_crosstable_NPNPIEONP2EOsum.mdb"

11.5.10. Click **Merge** Files. If either Unmatched Analytes or Unmatched Units appear in red, repair the cross table with the assistance, if necessary, of the Group Leader. Verify that the results in Initial Result are correct, and click **Save**, which will create an Excel file. Name this one differently from the name chosen previously and click **Done**.

11.5.11. Return to the **Data Entry/Review** module and click **Open**, using the .xls file created in the paragraph above. Verify that all items are correct as in the manual data entry in 11.5.3 and click **Save**. Query the data and proceed as in 11.5.4.

11.6. LIMS Report Generation

11.6.1. Once all LC/MS data is entered with the status of analyzed and the data is locked, prepare a draft report. In LIMS, select Project Management, Reports. Choose the work order and the analyses, and select the report. For LC/MS analyses, the LIMS analyses are all multiple element analyses as of this writing. The report chosen will typically be C_Analysis_v2.rpt. with no ISO 17025 Logo. If QC is to be omitted from the report, choose Modified Draft, unchecking the quality control samples that were not analyzed. This draft report need not be signed. It is only for the purpose of review.

11.6.2. After the data reviewer has updated the status of the LIMS entries to Reviewed, the final report may be generated. The mode of generation of the report is the same as above, and either the Final Report or Modified Final Report is chosen. The first page of the report must be signed and dated.

12. Trouble Shooting

12.1. Symptom: Inadequate Abundance or Sensitivity

Probable Causes:

12.1.1. Dirty or contaminated ion source, electron multiplier, or quadrupole rod surfaces.

12.1.2. Potentials of ion source elements at wrong values due to open or short circuits.

12.1.3. Faulty ion source electronics, detector electronics or power supply.

12.2. Symptom: Improper Isotope Ratios

Probable Causes:

12.2.1. High "background" levels of undesired substances (Contamination from earlier sample injections) which contribute additional abundance at the isotope mass. Clean the ion source assembly and condition the column.

12.2.2. Resolution of adjacent masses set improperly: higher than normal ratios due to poor resolution (peaks too wide) or lower ratios due to over resolution (narrow peaks).

12.3. Symptom: High Background

Probable Causes:

12.3.1. Dirty or contaminated ion source, electron multiplier or quadrupole rod surfaces.

12.3.2. "Yesterday's" Samples - There is the possibility that some previously injected sample can still be present in the vacuum system long after it was thought to be evacuated. This phenomenon depends on sample volatility, temperature, etc.

12.3.3. Contamination in a recently cleaned vacuum system - After any venting of a vacuum system for maintenance, there is the potential for introducing new substances into the vacuum system. Some substances are normal and can be pumped out, while others require more cleaning or baking.

12.4.3.1. Solvents used in the cleaning process: These will be present for a while but should be pumped out of the vacuum system.

12.4.3.2. Water absorbed on the metal surfaces while vented. This will be pumped out.

12.4.3.3. "Fingerprints" - Heavy organic substances from inadequate clean room procedures may not be pumped out and may require source cleaning.

12.4. Symptom: Mass Spectrometer Does Not Respond

Probable Cause:

12.4.1. The mass spectrometer electronics are not on - Check the switch.
12.4.2. Secondary fuse blown - Check the secondary fuses on the rear of the mass spectrometer and replace the faulty fuse or fuses.
12.4.3. Board failure
12.4.4. Communication loss, reboot mass spectrometer, PC and LC.

13. Preventative Maintenance

Log book for record of preventative maintenance and repair work of LC-MS is located near the instrument, entries are dated and initialed.

Instrument manual is located on the instrument computer associated with the instrument software.

14. References

Code of Federal Regulations, 40 CFR Part 136, Appendix B.

Standard Practices for Preparation of Sample Containers and for Preservation of Organic Constituents, American Society for Testing and Materials, Philadelphia. ASTM Annual Book of Standards, Part 31, D3694-78.

Carcinogens - Working with Carcinogens; U.S. Department of Health, Education and Welfare. Center for Disease Control. National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

OSHA Safety and Health Standards, General Industry, (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).

Standard Practices for Sampling Water, American Society for Testing and Materials, Philadelphia. ASTM Annual Book Standards, Part 31, D3370-76.

McNair, N.M.; Bonelli, E.J. *Basic Chromatography*;, Consolidated Printing: Berkeley, CA, 1969; p. 52.

Olynyk, P.; Budde, W.L.; Eichelberger, J.W. "Method Detection Limit for Methods 624 and 625", Unpublished report, October 1980.

15. Revision History

Revision #	Status	Description
0.0	I	This document is first version of the SOP for analysis of TTPC in water.

I = Initial, R = Revision, A = Archived

APPENDICES

Tables and Validation Data

Item	Title	Number of Pages	Revision Number	Date Revised
Table 1	Method Parameters	1	0	July 2014
Table 2	Quality Control Acceptance Criteria and Uncertainty	1	0	July 2014
Table 3	Concentration of Calibration Standards	1	0	July 2014
Table 4	Preparation of Calibration Standards	1	0	July 2014
Table 5	Retention Times and SRM Ions	1	0	July 2014
Table 6	Gradient Conditions for Liquid Chromatography	1	0	July 2014
Table 7	Variable Mass Spectrometer Parameters Depending on Analyte	1	0	July 2014
Table 8	MDL Study	1	0	July 2014
Table 9	Precision and Accuracy Study in ASTM Type I Water and River Water	1	0	July 2014

TABLE 1.

OM16- Method Parameters

Analyte	MDL (ng/L)	Reporting Ranges (ng/L)
TTPC	13.11	100 - 4000

*Method Detection Limits will be updated regularly whenever data is to be reported between the MDL and RL. Last MDL Study- July 2014, CRL work order 1408005. MDL is calculated based upon nine spiked samples.

TABLE 2. Quality Control Acceptance Criteria and Uncertainty

PARAMETER	Average Recovery (%)	Standard Deviation (%)	# of Replicates (n)	Lower Control Limit (LCL) %	Upper Control Limit (UCL) %	Uncertainty (95% Confidence Interval)
TTPC	94.66	2.26	6	87.9	101.4	2.37

*The quality control acceptance criteria shall be updated regularly. Last Study- July 2014, CRL work order 1408005.

**** Uncertainty calculation based upon 95% Confidence Interval and a 2-tailed student t distribution. Uncertainty = Student t Value ((Standard Deviation/(Number of LCS)$^{1/2}$)**

TABLE 3. Concentrations of Calibration Standards (ng/L)

Concentrations (ng/L)	**LV1**	**LV2**	**LV3**	**LV4**	**LV5**	**LV6**	**LV7**	**LV8**
TTPC	25	50	100	200	400	600	800	1000

TABLE 4. Preparation of Calibration Standards

Solution	LV1	LV2	LV3	LV4	LV5	LV6	LV7	LV8
A	25 µL	50 µL	100 µL	200 µL	400 µL	600 µL	800 µL	1000 µL
B	975 µL	950 µL	900 µL	800 µL	600 µL	400 µL	200 µL	0 µL

Solution A: Level 8 stock solution prepared according to Section 7 and at Table 4 concentrations.

Solution B: 75% Acetone:25% Water

TABLE 5. Retention Time (RT) and SRM Ions

Chemical	Primary/Confirmatory	SRM Transition	Retention Time (minutes)	Primary/ Confirmatory SRM Area Ratio
TTPC	Primary (Quantitation)	399.5 → 229.3	8.1	NA
	First Confirmatory	399.5 →75.9		0.92
	Second Confirmatory	399.5 → 343.5		3.02

TABLE 6. Gradient Conditions for Liquid Chromatography

Time (min)	Flow (mL/min)	95%Water: 5%Acetonitrile	Acetonitrile	400mM ammonium acetate (95% water: 5% Acetonitrile)
0	0.3	95	0	5
1	0.3	95	0	5
4	0.4	0	95	5
11	0.4	0	95	5
12	0.4	95	0	5
15	0.4	95	0	5

TABLE 7. Variable Mass Spectrometer Parameters

Chemical	Primary/Confirmatory	SRM Transition	Cone (V)	Collision (eV)
TTPC	Primary	399.5 → 229.3	40	45
	First Confirmatory	399.5 →75.9	40	46
	Second Confirmatory	399.5 → 343.5	40	40

TABLE 8. MDL Study (July 2014, CRL Work Order 1408005)

Analyte	Spike Conc. (ng/L)	Mean Recovery (ng/L)	Mean Recovery (%)	RSD (%)	Standard Deviation	MDL (ng/L)
TTPC	100	99.5	99.5	4.54	4.52	13.11

TABLE 9. Precision and Accuracy Study in ASTM Type I Water and Chicago River Water (CRW) (July 2014)

Sample	**ASTM P&A Data (2000 ng/L spike)**	**CRW P&A Data (2000 ng/L spike)**
	TTPC	**TTPC**
MB 1	<RL	<RL
MB 2	<RL	<RL
P&A 1	1861	1898
P&A 2	1891	1965
P&A 3	1882	1933
P&A 4	1894	1871
P&A 5	1975	1929
P&A 6	1857	1974
Average Recovery (ng/L)	1893	1928
% Average Recovery	94.6	96.4
Standard Deviation	42.8	39.1
RSD (%)	2.26	2.03